Filing Date: 5 June 1995 Serial No.: 08/465,322

Page 3

be located in the target polymer in any position between the position of the primer-end complement nucleotide and the predetermined target position;

- (b) an enzymatic polymerizing agent; and
- (c) an admixture of nucleoside triphosphates including at least one deoxynucleotide and at least two chain-terminating nucleotide analogues, at least one deoxynucleotide comprising a detectable label or an attachment moiety capable of binding a detectable label,

so that in use the detection primer can hybridize to the target nucleic-acid polymer at the primer-hybridizing nucleotide sequence and form a detection-primer extension product by an enzyme-catalyzed primer-extension reaction to permit the presence or absence of a specific nucleotide at the predetermined target position to be detected by detecting the presence or absence of a corresponding detectable label in association with the detection-primer extension product.

- 98. (New) A reagent kit according to claim 97 wherein the detection primer comprises an attachment moiety.
- 99. (New) A reagent kit according to claim 97 wherein the detection-primer nucleotide sequence is from 10 to 40 nucleotides in length.
- 100. (New) A reagent kit according to claim 97 wherein each chain-terminating nucleotide analogue of the nucleoside triphosphates of paragraph (c) is a dideoxyribonucleotide selected from the group consisting of ddATP, ddGTP, ddCTP, and ddTTP.

Applicants: Soderlund, Hans E., and Syvanen, Anne-Christine Filing Date: 5 June 1995 Serial No.: 08/465,322 Page 4 A reagent kit according to claim 97 wherein the nucleoside triphosphates of paragraph (c) include at least two deoxynucleotides, at least one of which deoxynucleotide comprises a detectable label or an attachment moiety capable of binding a detectable label. A reagent kit according to claim 97 wherein each deoxynucleotide of the 102. (New) nucleoside triphosphates of paragraph (c) is a deoxyribonucleoside triphosphate selected from the group consisting of dATP, dGTP, dCTP, dUTP, and dTTP. A reagent kit according to claim 97 in which the primer-end complement 103. (New) nucleotide is located in the target nucleic-acid polymer at a position immediately adjacent to the predetermined target position. A reagent kit according to claim 97 in which the detectable label is a radioisotope. 104. (New) 105. (New) A reagent kit according to claim 97 further comprising: a pair of amplification primers for amplifying the target nucleic-acid polymer, the (d) two amplification primers bracketing the predetermined target position in the target polymer, at least one of the amplification primers comprising an attachment moiety for immobilizing target nucleic-acid polymer molecules on a solid support. 106. (New) A reagent kit according to claim 105 further comprising: (e) a solid support. 107. (New) A reagent kit for detecting the presence or absence of one or more specific

nucleotides at a predetermined target position in a target nucleic-acid polymer, comprising:

Filing Date: 5 June 1995 Serial No.: 08/465,322

Page 5

- (a) a detection primer comprising a detection-primer nucleotide sequence having a primer-extension-initiation 3'-end nucleotide which constitutes a 3' terminal end of the detection primer, the detection-primer nucleotide sequence being complementary to a primer-hybridizing nucleotide sequence of the target nucleicacid polymer with a nucleotide in the target nucleic-acid polymer complementary to the primer-extension-initiation 3'-end nucleotide of the detection-primer nucleotide sequence defining a primer-end complement nucleotide, the primerhybridizing nucleotide sequence of the target nucleic-acid polymer extending towards the 3' end of the target polymer from the primer-end complement nucleotide, the primer-end complement nucleotide being located in the target polymer at a position 3'-ward of the predetermined target position, the position of the primer-end complement nucleotide being subject to a constraint that no nucleotide of the same type as the one or more specific nucleotides to be detected be located in the target polymer in any position between the position of the primer-end complement nucleotide and the predetermined target position;
- (b) an enzymatic polymerizing agent; and
- (c) an admixture of nucleoside triphosphates including at least one deoxynucleotide and at least one chain-terminating nucleotide analogue, at least one chain-terminating nucleotide analogue comprising a detectable label or an attachment moiety capable of binding a detectable label, each deoxynucleotide of the admixture of nucleoside triphosphates being complementary to a nucleotide which differs from any nucleotide to which a chain-terminating nucleotide analogue of the admixture is complementary;

Filing Date: 5 June 1995 Serial No.: 08/465,322

Page 6

so that in use the detection primer can hybridize to the target nucleic-acid polymer at the primer-hybridizing nucleotide sequence and form a detection-primer extension product by an enzyme-catalyzed primer-extension reaction to permit the presence or absence of a specific nucleotide at the predetermined target position to be detected by detecting the presence or absence of a corresponding detectable label in association with the detection-primer extension product.

108. (New) A reagent kit according to claim 107 wherein the detection primer comprises an attachment moiety.

109. (New) A reagent kit according to claim 107 wherein the detection-primer nucleotide sequence is from 10 to 40 nucleotides in length.

110. (New) A reagent kit according to claim 107 wherein each chain-terminating nucleotide analogue of the nucleoside triphosphates of paragraph (c) is a dideoxyribonucleotide selected from the group consisting of ddATP, ddGTP, ddCTP, and ddTTP.

111. (New) A reagent kit according to claim 110 in which at least one dideoxyribonucleotide of the nucleoside triphosphates of paragraph (c) comprises a detectable label consisting of a fluorescent group.

112. (New) A reagent kit according to claim 107 wherein the nucleoside triphosphates of paragraph (c) include at least two deoxynucleotides.

113. (New) A reagent kit according to claim 107 wherein each deoxynucleotide of the nucleoside triphosphates of paragraph (c) is a deoxyribonucleoside triphosphate selected from the group consisting of dATP, dGTP, dCTP, dUTP, and dTTP.

Filing Date: 5 June 1995 Serial No.: 08/465,322

Page 7

114. (New) A reagent kit according to claim 107 in which the primer-end complement nucleotide is located in the target nucleic-acid polymer at a position immediately adjacent to the predetermined target position.

115. (New) A reagent kit according to claim 107 further comprising:

(d) a pair of amplification primers for amplifying the target nucleic-acid polymer, the two amplification primers bracketing the predetermined target position in the target polymer, at least one of the amplification primers comprising an attachment moiety for immobilizing target nucleic-acid polymer molecules on a solid support.

116. (New) A reagent kit according to claim 115 further comprising:

(e) a solid support.

REMARKS

A. Summary of the Invention

Broadly, the present invention concerns a reagent kit for detecting the presence or absence of one or more specific nucleotides at a predetermined target position in a target nucleic-acid polymer.

The reagent kit includes a detection primer comprising a detection-primer nucleotide sequence having a primer-extension-initiation 3'-end nucleotide which constitutes a 3' terminal end of the detection primer. The detection-primer nucleotide sequence is complementary to a primer-hybridizing nucleotide sequence of the target nucleic-acid polymer with a nucleotide in